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Phyto-chemical analysis of peruvian medicinal plants

Análisis fitoquímico de plantas medicinales peruanas

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Abstract

This study reports on the chemical compound composition of 78 species of plants traditionally used as medicines on the North coast of Peru. This first assay serves as a baseline for more in-depth studies on plant compound composition and serves as background to understand and better apply bio-assays results.

Key words: Phyto-chemical analysis, medicinal plants, northern Peru.

Resumen

Este estudio reporta sobre la composición de compuestos químicos en 78 plantas tradicionalmente usadas como medicina en la región de costa norte de Perú. Este ensayo preliminar sirve como base para estudios mas avanzados sobre composición química de plantas medicinales, y también como fondo para entender y mejorar bio-ensayos.

Palabras clave: Análisis fitoquímico, plantas medicinales, norte del Perú.

Introduction

The Minority Health International Research Training Program (MHIRT) conducted in cooperation between Universidad Antenor Orrego and Clinica Anticona in Trujillo, Peru and San Diego State University, University of California Berkeley and Missouri Botanical garden in the US, began in the summer of 2005 to explore the bioactivity of medicinal plant species documented previously. Very few studies conducted bioassays on Andean medicinal plants (Neto *et al.* 2002, Muñoz & Sauvain 2002, Bussmann *et al.* 2008).

Although phytochemistry and ethnbotany in combination are demonstrating the need for preservation and validation of traditional herbal use, in general not enough research is being done in phytochemistry, especially projects that link local herbal specialists and traditional healers with phytochemists and medical ethnobotanists. Nothing at all has been published about the possible chemical compound composition responsible for the bioactivity of the local medicinal flora. Our ongoing project undertakes such a task using the science paradigm united with traditional cultural practices to validate and further explain the properties of herbs that have been used for hundreds of years. To allow easy repeatability we provide a short testing manual.

Materials and Methods

Experimental design

Five hundred and twelve species of medicinal plants were collected in the Peruvian departments Lambayeque and La Libertad in the field, in markets of the coastal cities Trujillo and Chiclayo and around the homes and in gardens of traditional healers (*curanderos*) of the region. Fieldwork was conducted in August-September 2001, July-August 2002, July-August 2003, June-August 2004, July-August 2005, July-August 2006, June-August 2007 and June–August 2008. For an exact description of the collection methodology see Bussmann &b Sharon. (2006). The selected samples are identified and botanically classified. Then, they are dried in shade or at 40ÚC and pulverized.

Spot tests were used to allow to qualitatively to determine the type of metabolites present in a vegetal extract. The method consists of submitting the vegetal extracts to specific reagents that generate colored compounds (Dominguez, 1973; Harborne, 1984; Trease, 1986; Barba, 1997).

Extract preparation with solvents of different polarities

a. Weigh three samples of dried and ground vegetal material (2.5g; 2.5g; and 5.0g), package them in filter paper and place them in 200 ml beakers.

b. Add 30ml of solvent (chloroform, ethanol, and water) and cover with a watch glass.

c. Submit to heating with a hot water bath for 10 minutes, avoiding the complete evaporation of the solvent.

d. Carry out the qualitative spot tests

e. To the beaker that contains the water extract (free of the extract), add 5% HCl and carry out steps c and d.

Tests

The common tests to identify secondary metabolites are:

Foam:	saponins
Ferric chloride or gelatin:	tannins
Shinoda:	flavonoids
Liebermann-Burchard:	steroids
Borntraeger:	quinones
Dragendorff:	alkaloids
Meyer:	alkaloids
Wagner:	alkaloids
Kedde:	cardiotonics
pH	anthocyans

Dichloromethane or chloroform extracts

1. *Steroids:* Liebermann-Burchard test. To 5 drops of extract, add 5 drops of acetic anhydride and then 2 drops of sulfuric acid concentrate. A green, blue, blue green, violet or red coloration indicates the presence of nucleo steroid or triterpenoid.

2. *Quinones*: Borntrager test. To 5 drops of dried extract, add 5 drops of toluene (dissolved) and then add 5 drops of 5% NaOH. The appearance of a red coloration in the aqueous phase indicates the presence of antiquinones and naftoquinones.

Alternative method

Boil two grams of the pulverized plant with 10 ml of 5% KOH and 10 ml of 6% hydrogen peroxide for 5 minutes and cool down. Separate the liquid extract and acidulate with 5 ml glacial acetic acid, to later carry out an extraction with benzene. The benzene cap becomes yellow. It is separated and 5 ml of that solution are agitated with NH_4OH. The antiquinones color the alkaline cap red.

Methanolic or ethanolic extract

1. *Steroids:* To 5 drops of dried extract, add 5 drops of dichloromethane or chloroform (dissolved) and carry out the Liebermann-Burchard test.

2. *Flavonoids:* Shinoda test, add 5 drops of sample to a few pieces of metallic magnesium and then add 3 drops of acidic chloride concentrate. A reddish coloration indicates the presence of flavonoids.

3. *Cardiotonics:* Kedde test. To 5 drops of extract, add 3 drops of the keede reactive. The appearance of violet or purple coloration is a positive test for the existence of cardiotonics.

4. *Tannins:* Ferric chloride or gelatin test. To 5 drops of extract, add two drops of 10% $FeCl_3$ solution. A blue coloration indicates the presence of hydrolysable tannins and a green coloration indicates the presence of condensed tannins.

Aqueous Extract

1. *Anthocyans:* pH test (acid and basic medium). To 5 drops of extract, add 3 drops of HCl concentrate. Observe the color formed. To another sample of 5 drops of extract, add 3 drops of 5% NaOH. Observe the color formed. The anthocyans are known for their ability to produce different colors at a different pH.

2. *Saponins:* Foam test. Place 3 ml of the extract in a test tube and agitate vigorously for 30 seconds, wait 15 minutes. The persistence of the foam indicates the presence of saponins.

3. *Tannins:* Ferric chloride or gelatin test.

Acidic Extract (5% HCl)

1. *Alkaloids:* Dragendorff, Meyer, and Wagner test so place in 3 test tubes, 1 ml of acidic extract. Add to each one, two drops of the reagents Dragendorff, Mayer, and Wagner. If turbidity is observed or precipitates (red to orange, white to cream and brown), the sample is considered to contain alkaloids.

Results

Table 1 gives an overview on the plants tested and the secondary metabolites that they contained. The study conducted a phyto-chemical analysis on a total of 78 plants. Six (7.69 %) out of the 78 plants contained alkaloids. A total of 36 (46.75 %) plants tested that contained flavonoids, 40 (51.95 cardiotonics, 12 (17.39 %) contained saponins, .62 (80.52 %) plants contained tannins, 6 (8.0 %) quinones, 68 (90.67 %) steroids, and. 64 (82.05 %) plants contained anthocyans.

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Table1: Plants tested and their compounds

		Secondary Metabolites							
		Alkaloids	Flavonoids	Cardiotonics	Saponins	Tannins	Quinones	Steroids	nthocyans
Scientific name	Common name	HCl	EtOH	EtOH	H2O	EtOH	ChCl3	ChCl3	H2O
Acanthaceae									
Aphelandra cirsioides Lindau	Espina de Hoja	N	P	P	N	P	N	?	P
Adiantaceae									
Adiantum concinnum Willd.	Culantrillo	N	N	P	N	N	N	P	P
Adiantum concinnum Willd.	Culantrillo de Pozo	N	P	P	N	P	N	P	P
Amaranthaceae									
Amaranthus hybridus L.	Yuyo	N	N	N	N	N	N	N	N
Anacardiaceae									
Loxopterygium huasango Spruce ex Engl.	Hualtaco	P	N	P	P	P	N	N	P
Apiaceae									
Niphogeton dissecta J.F. Macbr.	Omamo Motora	N	P	P	N	P	N	P	P
Petroselinum crispum (Miller) A.W. Hill	Perejil	N	P	N	N	P	N	P	N
Pimpinella anisum L.	Anis	N	N	N	N	N	N	P	N
Apocynaceae									
Mandevilla antennacea K. Schum.	Bejuco Colambo Negro	P	N	N	?	?	N	P	N
Aristolochiaceae									
Aristolochia ruiziana Duch.	Bejuco Contr. Aire	N	N	P	N	P	N	P	P
Asteraceae									
Ambrosia peruviana Willd.	Ajenjo, Ajenco	N	N	N	N	N	N	P	P
Arctium lappa L.	Lampazo	N	N	P	N	P	N	P	P
Baccharis latifolia Pers.	Chilca Chica	N	N	P	P	P	N	P	P
Baccharis latifolia Pers.	Chilca Grande	N	N	N	N	P	N	P	P
Baccharis odorata H.B. & K.	Pasto Miel	N	N	N	P	P	N	P	P
Baccharis salicifolia (Ruiz. & Pav.) Pers.	Hierba de la Plata	N	P	N	N	P	N	P	N
Clibadium sylvestre (Aubl.) Baill.	Flor de Novia N	P	N	N	P	N	P	P	
Clibadium sylvestre (Aubl.) Baill.	Rosa de Novia	N	P	P	N	P	N	P	P
Cynara cardunculus L.	Alcachofa	N	N	N	N	P	N	P	P
Eupatorium triplinerve Vahl.	Chilco Hembra	N	N	N	N	N	N	P	P
Senecio chionogeton Wedd.	Hórnamo León Amarillo	N	P	P	N	P	N	P	P
Senecio hypsiandinus Cuatrec.	Órnamo Blanco	N	N	P	N	P	N	P	P
Tagetes erecta L.	Flor de Muerto	N	P	P	N	P	N	P	P
Bignoniaceae									
Cydista aequinoctialis Miers	Bejuco Amarillo	N	N	N	N	N	N	P	N
Brassicaceae									
Brassica oleracea L. ssp. *capitata*	Hoja de Col	N	P	N	N	P	N	P	P
Berberidaceae									
Berberis buceronis J.F. Macbr.	Palo Amarillo N	P	P	P	P	N	?	P	
Bixaceae									

Bixa orellana L.	Achiote	N	N	P	N	P	N	P	P
Boraginaceae									
Cordia alliodora (Ruiz. & Pav.) Oken	Ajo Sacha	N	N	N	N	N	N	P	N
Burseraceae									
Bursera graveolens Triana & Planch.	Palo Santo	N	P	P	N	P	N	P	P
Chenopodiaceae									
Chenopodium quinoa Willd. (silvestre)	Quino Amargo	P	N	N	?	N	N	P	N
Convolvulaceae									
Ipomea pauciflora M. Martens & Galeotti	Huanarpo	N	P	P	N	P	N	P	P
Cusiaceae									
Clusia minor L.	Chusgón	N	P	P	P	P	P	P	P
Hypericum silenoides Juss.	Cintaura	N	P	N	N	P	N	N	P
Fabaceae									
Melilotus alba Medik.	Alfalfilla	N	P	N	N	P	N	P	N
Prosopis pallida (Humb. & Bonpl. ex Willd.) Kunth	Algarrobo	P	P	P	P	P	N	P	P
Senna bicapsularis (L.) Roxb.	Alcaparilla	N	N	P	N	P	P	P	P
Senna occidentalis (L.) Link.	Ratania	N	P	P	P	P	P	P	P
Lamiaceae									
Lepechinia meyenii (Walp.) Epling	Sálvia Real	N	N	P	?	P	N	P	P
Lippia integrifolia Hieron	Poleo del Inca	N	P	P	N	P	N	P	P
Mentha piperita Stokes	Poleo	N	P	P	N	P	N	P	P
Salvia cuspidata Ruiz. & Pav.	Sálvia Blanca	N	P	P	N	P	P	P	P
Salvia sagitatta Ruiz. & Pav.	Sálvia Negra N	P	P	N	P	P	P	P	
Stachys lanata Jacq.	Veronica (Macho)	N	P	N	N	P	N	P	P
Thymus vulgaris L.	Tomillo	N	P	P	N	P	N	P	P
Lauraceae									
Nectandra floribunda Nees	Ishpingo	N	P	N	N	P	?	P	P
Lecytidaceae									
Gustavia augusta L.	Chope	N	N	N	?	N	N	P	P
Lobeliaceae									
Centropogon articulatus Drake	Conchalalay	N	N	N	?	P	N	P	P
Malesherbiaceae									
Malesherbia ardens J.F. Macbr.	Veronica	N	N	N	N	P	N	P	P
Melastomataceae									
Brachyotum tyrianthinum Macbr.	Sarcilleja	N	N	P	N	P	N	P	P
Menispermaceae									
Abuta grandifolia (Mart.) Sandwith	Abuta	P	N	N	N	P	N	N	N
Moracea									
Ficus sp.	Higuerón	N	N	N	N	N	N	P	P
Myrtaceae									
Myricanthes fragrans (Sw.) McVaughn	Lanche	N	N	P	P	P	N	P	P

Psidium guayava L.	Guanábana	N	P	P	N	P	N	P	P
Syzygium jambos (L.) Alston	Poma Rosa	N	P	P	N	P	N	P	P
Olacaceae									
Ximenia americana L.	Limoncillo	N	?	?	N	P	?	?	P
Papaveraceae									
Argemone mexicana L.	Cardo Santo	N	N	N	N	N	N	P	P
Phytolaccaceae									
Gallesia integrifolia (Spreng.) Harms.	Palo de Ajo	N	N	N	N	P	P	P	P
Plantaginaceae									
Plantago major L.	Llantén	N	N	N	N	P	N	P	P
Plantago sericea Ruiz. & Pav. ssp. *sericans* (Pilger) Rahn	Paja Blanca	N	N	N	N	P	N	P	P
Poaceae									
Hordeum vulgare L.	Cebada	N	P	N	N	N	N	P	N
Polemoniaceae									
Cantua buxifolia Lam.	Cantu	N	P	N	P	P	N	P	P
Ranunculaceae									
Thalictrum decipiens B. Boivin	Chontilla (Chica)	N	N	N	N	P	N	P	P
Rosaceae									
Prunus serotina Ehrh.	Helialiso	N	N	P	N	P	N	P	P
Prunus serotina Ehrh. subsp. *capuli* (Cav.) McVaugh	Capuli	N	P	P	N	P	N	P	P
Rubiaceae									
Uncaria tomentosa DC.	Uña de Gato	N	N	P	N	P	N	N	P
Rutaceae									
Citrus sinensis Osbeck	Naranja	P	N	N	?	P	N	P	P
Smilacaceae									
Smilax kunthii Killip & Morton	Palo de la China	N	P	N	N	P	?	P	P
Solanaceae									
Capsicum chinense Jacq.	Aji Panca	N	P	N	N	P	N	P	N
Capsicum rhomboideum (Kunth) Kunze.	Aji Colorado	N	N	P	?	P	N	P	N
Cestrum strigilatum Ruiz. & Pav.	Santa Maria	N	N	P	N	P	N	P	P
Lycopersicon hirsutum Dunal	Ambulluco de Muerto	N	N	P	N	P	N	P	P
Solanum tuberosum L.	Chuño de Papa	N	N	N	N	N	N	P	N
Urticaceae									
Urtica urens L.	Ortiga	N	P	N	?	N	N	P	

N= negative, P=compound present